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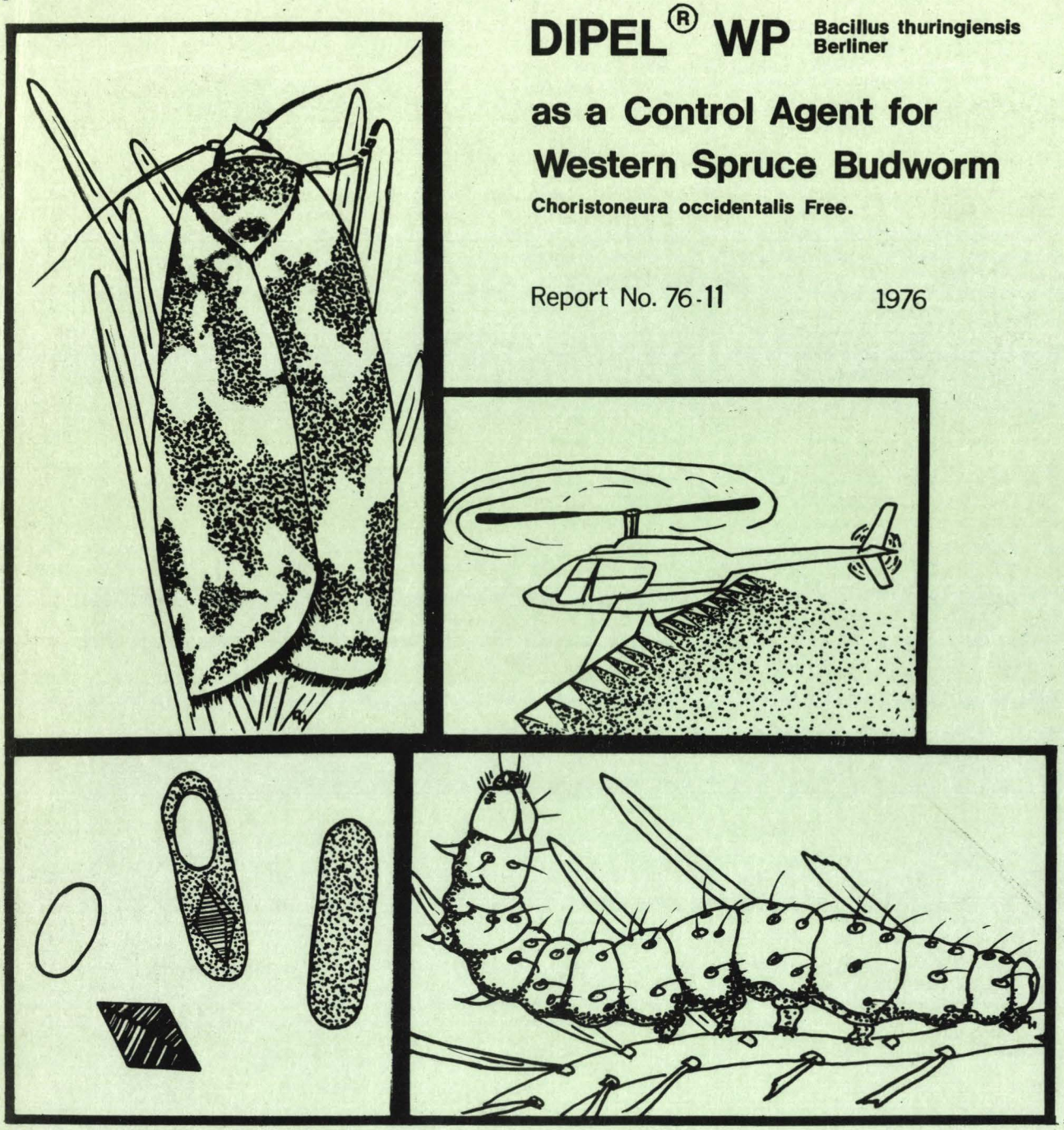

DIPEL® WP

Bacillus thuringiensis Berliner

as a Control Agent for Western Spruce Budworm

Choristoneura occidentalis Free.

Report No. 76-11 1976

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Cover: Diagrammatic representation of western spruce budworm-*B.t.* pilot project phases. Clockwise: Budworm adult, spray ship, budworm larva, and *Bacillus thuringiensis* cells.

DIPEL(R) WETTABLE POWDER (*Bacillus thuringiensis* Berliner) AS A CONTROL AGENT FOR WESTERN SPRUCE BUDWORM *Choristoneura occidentalis* Free.

By

M.D. McGregor, D.R. Hamel, and R.C. Lood

ABSTRACT

Dipel(R) was applied by helicopter to three blocks of Douglas-fir and Engelmann spruce infested with *Choristoneura occidentalis* Free., in southwestern Montana. Dosage was 1 pound (7.2 BIU) in 2 gallons of water/acre (453 g in 7.5 ℓ/0.4 ha). A commercial surfactant, Bio-film(R) was added at the rate of 16 oz./100 gal. (453 g/378.5 ℓ) of spray. Rhodamine B extra S dye was added at the rate of 1.25 g/ℓ. Dipel was applied to mainly third and fourth instar budworm. Mortality, corrected by covariance analysis, at 21-day postspray counts indicated an average of 50.4 percent control. Additional mortality further reduced the population to an estimated 4.4 budworm/100 buds compared to 12 budworm/100 buds in check blocks. Foliage protection was 5.6 percent. Significant parasite population disruptions occurred following spray application.

INTRODUCTION

Dipel is a product of Abbott Industries. Active ingredient is the aerobic spore-forming bacterium, *Bacillus thuringiensis* Berliner. This pathogen is infective to numerous lepidopterous larvae. Dipel has been registered against such forest pests as tent caterpillars, fall webworm, gypsy moth, and elm spanworm. Dipel was selected for pilot testing on the Gallatin Ranger District because: (1) it was desirable to have a pesticide registered that could be applied in environmentally sensitive and high-use areas; (2) it was desirable to have more than one insecticide available, particularly if production of one pesticide was discontinued, an alternative would be available; (3) budworm may develop resistance to one pesticide; therefore, alternatives are needed; and (4) Dipel had

been tested in Canada and the United States and showed prospects of being effective and environmentally acceptable (Harper 1974; Klein and Lewis, 1966; Smirnoff *et al.* 1974; and Tripp 1971, 1973).

This test was designed to: (1) evaluate effectiveness of an aerial application of Dipel in reducing western spruce budworm populations under operational conditions; (2) measure effect of treatment in protecting foliage, both the year of treatment and the following year; and (3) determine effect of treatment on western spruce budworm parasites.

MATERIALS AND METHODS

Description of project areas.--The pilot project was conducted in Gallatin County, Montana (Figure 1), in an area where epidemic *C. occidentalis* populations have occurred since 1971 (Tunnock *et al.*, 1975). Douglas-fir stands within the Gallatin drainage have been subject to chronic annual defoliation by *C. occidentalis* since the early 1900's (Johnson and Denton 1975). Terrain is mountainous with elevations ranging from 5,400 to 7,800 feet (1,645 to 2,377 meters). Forest cover is a mixture of Douglas-fir, *Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco; Engelmann spruce, *Picea engelmannii* Parry; lodgepole pine, *Pinus contorta* Dougl.; and subalpine fir, *Abies lasiocarpa* (Hook Nutt. Douglas-fir and lodgepole pine occupy the majority of aspects on all sites. Spruce and subalpine fir are dominant in creek bottoms and draws. Stand age is about 90 years. Habitat type ranges from *Pseudotsuga menziesii/Linnaea borealis* to *Abies lasiocarpa/Linnaea borealis* (Pfister *et al.* 1974).

FORMULATION

The formulation Dipel WP, containing 7.2 BIU (Billion International Units) of the active aerobic spore-forming bacterium *Bacillus thuringiensis* was evaluated during this pilot test. Dipel was applied at the rate of 1 pound in 2 gallons of water/acre (453 g/7.5 ℓ/0.4 ha). A commercial surfactant Bio-film was added at 16 oz./100 gal. (453 g/378.5 ℓ) of spray. This material enhances Dipel by causing spray droplets to form a thin film on conifer needle surfaces rather than beading up. Rhodamine B extra S dye was added as a spray droplet tracer at the rate of 1.25 grams/liter.

PROJECT DESIGN

A randomized block experimental design was used in each area. Spray blocks were replicated three times each with a check block; each 1,075 to 1,220 acres (435 to 494 ha); and widely separated by prominent topographic features sufficient to minimize spray drift.

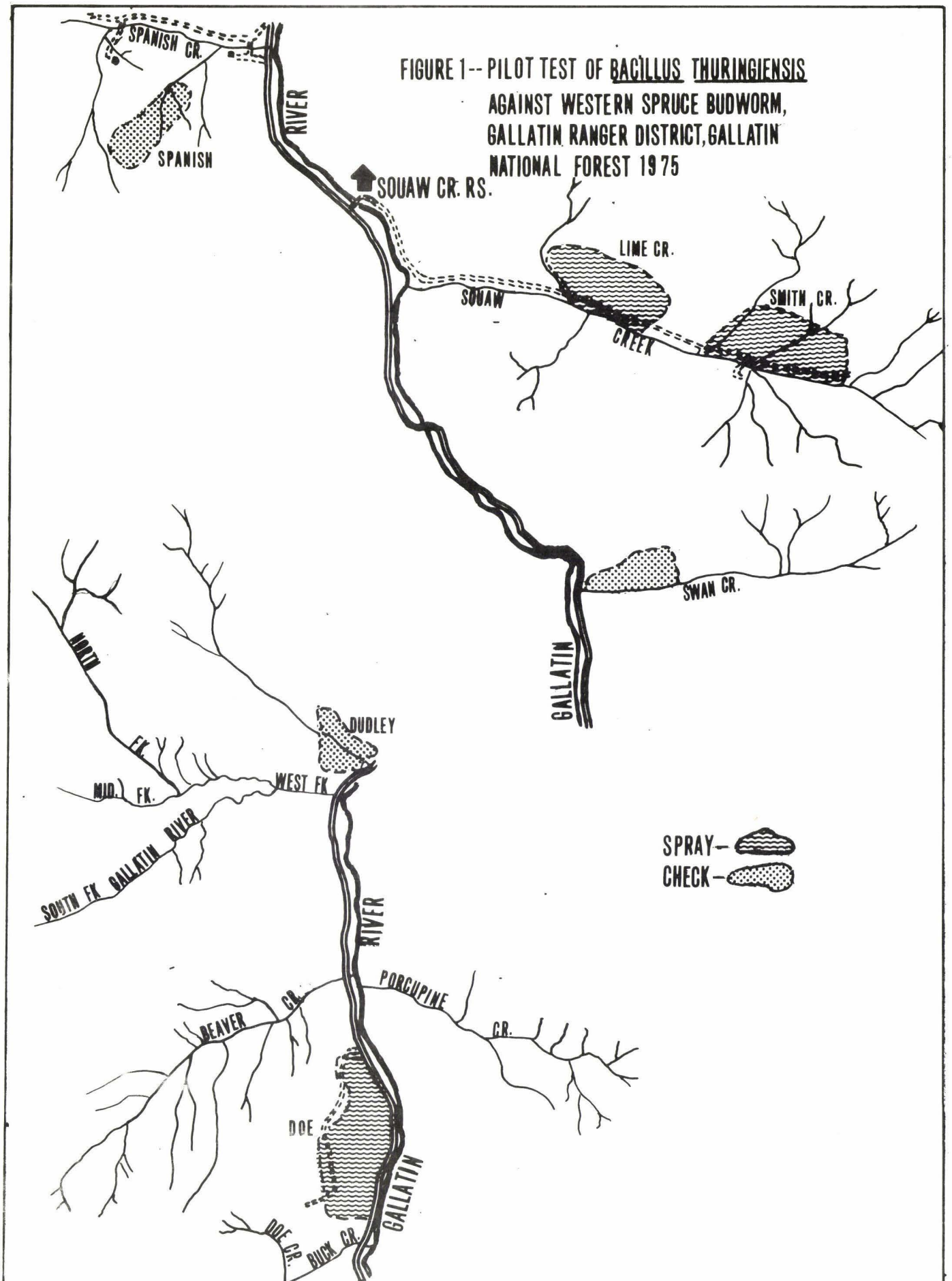


FIGURE 1-- PILOT TEST OF BACILLUS THURINGIENSIS AGAINST WESTERN SPRUCE BUDWORM, GALLATIN RANGER DISTRICT, GALLATIN NATIONAL FOREST 1975

Twenty-five clusters of three trees each (a total of 75 trees) of Douglas-fir or spruce were distributed throughout each block. Criteria for tree selection were that trees be 30 to 40 feet (9 to 12 m) tall, open grown, full crown, and accessible.

Budworm development sampling.--A critical part of the project was timing of spray application to coincide with larval development. Dipel was to be applied when 90 percent of the larvae were third and fourth instars. Shortly after diapause was broken, larval development was systematically measured by clipping two 15-inch (38-cm) branches from each of 20 trees from scattered locations in each treatment and check block. Branches were bagged and taken to the field laboratory where all larvae were removed and placed in 95 percent alcohol for instar determination. Instar determination was made by examining physical characteristics and by taking head capsule measurements. Development sampling was terminated when approximately 90 percent of the larval populations were third and fourth instars in Lime, Smith, and Doe Creek drainages.

Population sampling.--Prespray and postspray samples were collected by two-man crews raising telescopic pole pruners with catch bags attached into the midcrown of each sample tree and clipping the distal 15 inches (38 cm) in such a way that branches fell into the catch bag (Figure 2). The pole was lowered and sample material placed in a paper bag. Bags were stapled shut, labeled, and taken to the field laboratory. Two branches were collected per tree for prespray counts and four branches were collected for postspray assessment.

Figure 2.--Pole pruner sampling of larval spruce budworm populations.

Laboratory operations.--Branch samples were taken to cold storage the day of collection and examined within 48 hours of collection. Examiners, (Figure 3) counted number of buds/branch, and removed larvae, pupae, and parasites from foliage and placed them in Petri dishes (100 x 20 mm). An entomologist and trained technicians separated species of larvae. A maximum of 30 budworm larvae from each tree (10 per dish) were reared on artificial diet (modified after McMorran 1965). All parasites emerging from budworm were collected and placed in individual gelatin capsules (size 000). Recovered parasites were identified by specialists at the Insect Identification and Beneficial Insect Introduction Institute, Beltsville, Maryland. Other lepidopterous larvae found on samples were reared on artificial media for identification and to determine percent parasitism and parasite species present. Assessment of effects of Dipel on parasite populations was provided by conducting analysis of variance and "t" test to aid in making judgements concerning results.

Mixing and loading.--Dipel was mixed into solution in a 2,000-gallon (7,570 ℓ) tanker by personnel from Missoula Equipment Development Center, USFS, Missoula, Montana. The heliport for Lime and Smith Creek blocks was at Squaw Creek Ranger Station. The heliport for the Doe Creek block was at Porcupine Guard Station. Water used to mix Dipel was checked for pH prior to spraying and was determined to be between 6.3 and 7.

Spray application.--Dipel was applied with a 205-A Bell helicopter. Swath width at treetop height was 200 feet (61 m) at 90 m.p.h. (241 m/min.), at a release height of 50 feet (15 m) above treetops, depending on terrain. Nozzles were 8015 and tipped forward and down at 45°. Spraying started at 6 a.m. and finished at 10 p.m. each day. Spraying was not done when rain was forecast, or when temperatures exceeded 65° F (17° C). One block was sprayed each day. Each spray swath was marked on an aerial black and white photo. Tracking of swaths was done from a Bell 206 Jet Ranger helicopter. Constant radio communication was maintained between spray ship, chase ship, ground crews in spray blocks, and the loading site.

Spray deposit assessment.--Four white print-flex deposit cards were placed at cardinal directions at the drip-edge of the crown around each sample tree to assess spray deposit. An additional 50 cards were placed in an open area and 50 cards in a closed canopy in each spray plot. Cards were placed in plastic holders the morning prior to spraying, then picked up 1 hour after spraying. Assessment for percent deposit and volume median diameter (v.m.d.) of spray droplets was made by the Department of Defense, Dugway, Utah.

Figure 3.--Spruce budworm field laboratory operations; foliage examination (right), and species separations prior to rearing (below).

Assessment of treatment effect on foliage protection.--Amount of current year's foliage saved was measured by collecting four mid-crown branches from each sample tree after budworm pupation. Defoliation to the nearest 10 percent was measured on 25 apical shoots from each branch. Mean percent defoliation was determined for each tree. Linear regressions were computed with prespray population density as independent variable (x) and percent defoliation as dependent variable (y) for individual treatments. Covariance analysis was used to determine effects of treatment and if foliage was saved because of treatment by comparing Dipel treatment with check plots. This analysis provides an F test of adjusted means to determine significance.

RESULTS AND DISCUSSION

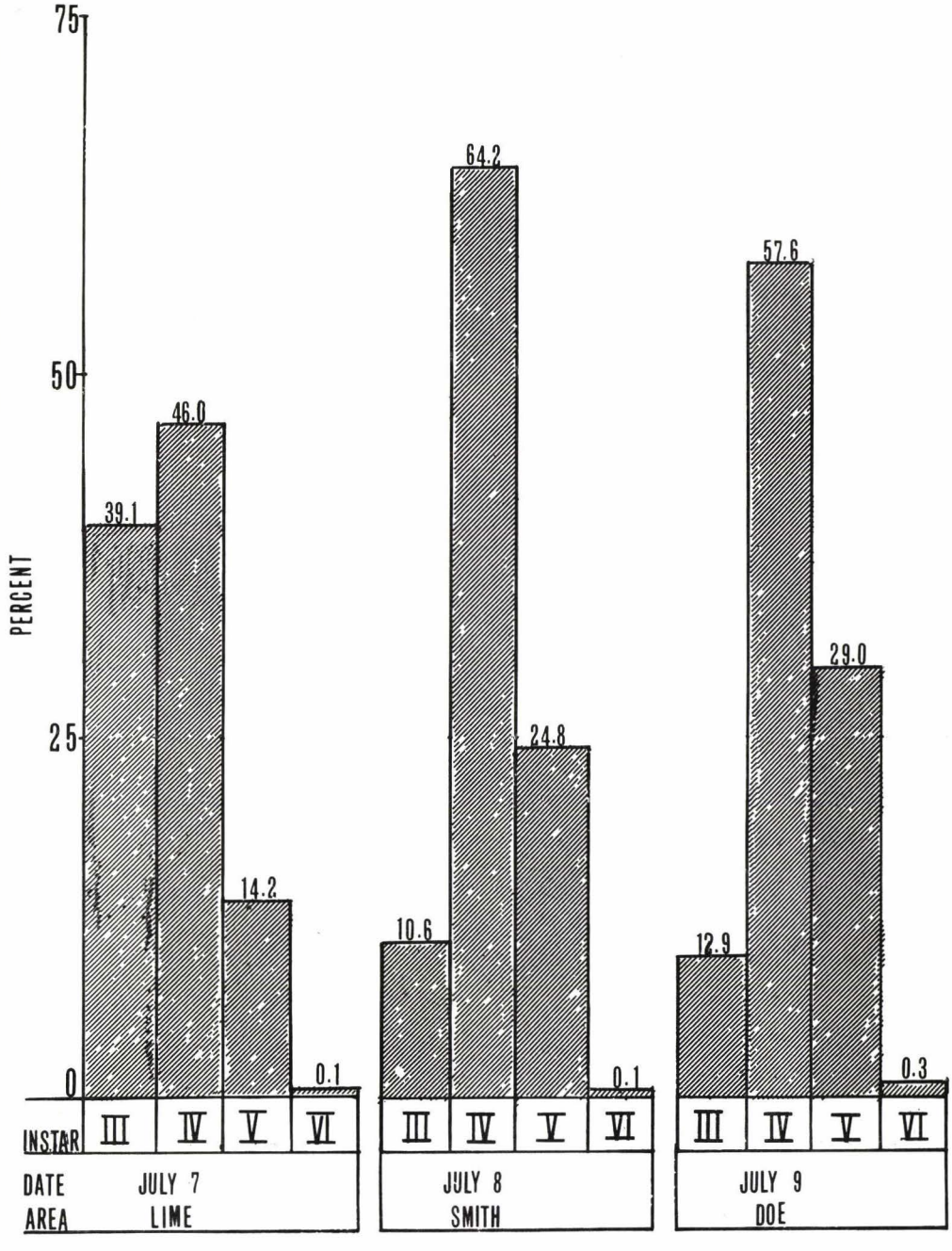
Lime, Smith, and Doe Creek blocks were treated on July 7, 8, and 9 between 6 a.m. and 10 a.m. respectively. Larvae were mostly third and fourth instars for all blocks the day of spraying (Figure 4). Best results have occurred where *B. thuringiensis* is applied when larval development is 90 percent third and fourth instars (Harper 1974). Local meteorological conditions were virtually optimum for aerial application of Dipel (i.e., air currents less than 6 m.p.h. (9.6 kms/hr) and temperatures below 65° F. (17° C.)) during spray operations. On July 7, when Lime Creek plot was sprayed, temperatures ranged from 55 to 65° F. (13 to 17° C.); relative humidity ranged from 60 to 80 percent; wind speed varied from 5 to 10 m.p.h. (8 to 16 kms/hr) mostly on ridgetops, with no precipitation or cloud cover. On July 8, when Smith Creek block was sprayed, temperatures ranged from 50 to 60° F. (10 to 16° C.); relative humidity ranged from 60 to 80 percent; wind speed was variable from 5 to 10 m.p.h. (8 to 16 kms/hr). Cloud cover developed throughout the morning. On July 9, when Doe Creek block was sprayed, temperature dropped, varying from 40 to 50° F. (4 to 10° C.); relative humidity ranged from 65 to 85 percent; wind speed was variable from 3 to 4 m.p.h. (5 to 7 kms/hr); and no precipitation occurred. Heavy fog moving in and out of the spray block made it necessary to constantly shift spray swaths.

Fog was so heavy at approximately 8 a.m. that spraying was stopped for approximately one-half hour. Moisture resulting from late afternoon and early evening squalls during spray operations was not sufficient to prevent adhesion of spray droplets to foliage.

Larval mortality.--Prespray population samples indicated substantial larval populations on both treatment and check blocks. Mean prespray population for all areas was 43 budworm larvae/100 buds. Population reductions and uncorrected percent control at 7-, 14-, and 21-day samples are shown in Table 1. Budworm populations in

FIGURE-4

LARVAL DEVELOPMENT FOR SPRAY BLOCKS, BACILLUS THURINGIENSIS PILOT TEST, GALLATIN RANGER DISTRICT, GALLATIN NATIONAL FOREST 1975



all three spray blocks were reduced to approximately 9 larvae/100 buds. Natural factors reduced populations in check blocks to approximately 19.6 larvae/100 buds. Through covariance analysis, corrected population control was determined (Table 2). Corrected percent control ranged from 32 percent in Doe Creek to 57 and 58 percent in Smith and Lime Creek blocks respectively.

In addition to mortality rates determined from field populations, an analysis of laboratory rearing data indicated that significant larval mortality also occurred in Dipel treated populations beyond the 21-day sample (Table 3).

Table 1.--Budworm population reduction, prespray to 21-day postspray, *Bacillus thuringiensis* pilot test, Gallatin Ranger District, Gallatin National Forest, 1975.

Block	Prespray population*	Postspray population 7-day	14-day	21-day
Spray				
Lime	42.5	26.4	17.8	8.8
% reduction		37.9	58.3	79.4
Smith	52.3	25.2	13.9	9.3
% reduction		51.9	73.5	82.3
Doe	31.7	21.6	15.7	9.5
% reduction		31.9	50.5	70.1
Check				
Spanish	50.4	44.6	44.5	23.1
% reduction		11.6	11.8	54.2
Swan	44.7	39.1	29.7	21.5
% reduction		12.6	33.6	52.0
Dudley	37.0	28.5	22.8	14.2
% reduction		23.0	28.4	61.7

*Expressed as number of budworm larvae per 100 buds.

Table 2.--Percent control, corrected by covariance analysis, by *Bacillus thuringiensis* against western spruce budworm, Gallatin Ranger District, Gallatin National Forest, 1975.

Area	Postspray population 7-day	14-day	21-day
Lime	36.23	57.28	58.09
Smith	38.46	56.71	57.05
Doe	20.73	29.33	31.96

Table 3.--Population surviving after 21-day postspray evaluation, *B.t.* pilot test, Gallatin Ranger District, Gallatin National Forest, Mt., 1976.

Treatments	Post 21-day evaluation 21-day pop./ 100 buds	Total % parasitism	Pop. after parasitism	% rearing mortality	Estimated pop. emerging/ 100 buds
B.t.	9.22	16.69	7.68	34.8	4.47
Check	19.65	15.33	16.63	21.8	12.34

The 13 percent difference in rearing mortality between treated and check blocks can reasonably be attributed to chronic *B.t.* infections which ultimately caused budworm mortality. As a result of this increased post 21-day mortality, an estimated 4.4 budworm per 100 buds would emerge to oviposit in treatment blocks. This compared to an estimated 12.3 larvae per 100 buds reinfesting check blocks. According to Harper (1974) this degree of population reduction by *B.t.* is sufficient to prevent additional defoliation damage the following year.

Parasite data.--This study showed that subsequent to application of Dipel significant parasite population disruptions occurred. Hamel (In Press) found 16 species of parasites attacking western spruce budworm (Table 4). Three weeks post treatment, significantly more *Apanteles fumiferanae* Vier. and *Glypta fumiferanae* (Vier.), and significantly fewer *Phaeogenes hariolus* (Cr.), *Ceromasia auricaudata* Tns., and *Madreymia saundersii* (Will.) and all other parasites occurred in spray blocks (Table 5). Hamel attributed apparent significant difference in parasitism by

Table 4.--Parasites recovered from *C. occidentalis*, Gallatin Ranger District, Gallatin National Forest, Montana, 1975.

Family and Species	Occurrence	Stage of host*	
		Attacked	Emerged from
Tachinidae			
Aplomya caesar (Ald.)	Rare	LL	LL, P
Ceromasia auricaudata Tns.	Common	LL	P
Madremyia saundersii (Will.)	Common	LL	LL, P
Braconidae			
Apanteles fumiferanae Vier.	Abundant	PL	LL
Meteorus campestris Vier.	Rare	LL	LL
Meteorus sp.	Rare	LL	LL
Ichneumonidae			
Ephialtes (=Apechthis) ontario (Cr.)	Rare	P	P
Exochus nigripalpis tectulum Townes	Rare	LL	LL, P
Gelis tenellus (Say.)	Rare	Hyperparasite	*A. fumiferanae*
Glypta fumiferanae (Vier.)	Abundant	PL	LL
Mesochorus tachypus Holm.	Rare	Hyperparasite	*A. fumiferanae*
Mesochorus sp.	Rare	Hyperparasite	*A. fumiferanae*
Phaeogenes hariolus (Cr.)	Abundant	P	P
Sinophorus sp.	Rare	?	?
Trichogrammatidae			
Trichogramma minutum Riley	Abundant	E	E
Pteromalidae			
Mesopolobus sp.	Common	Hyperparasite	*A. fumiferanae* *G. fumiferanae*

*E, PL, LL, P, and ? respectively refer to: egg, prewintering larva, late instar larva, pupa, and unknown.

Table 5.--Parasitism of *C. occidentalis* in *B.t.*-sprayed and check blocks, Gallatin Ranger District, Gallatin National Forest, Montana, 1975.

Sample period	Parasite species	No. *C. occidentalis* placed in rearing		Percent parasitism		Results of statistical testing
		B.t.	Check	*B.t.* Mean ± S.E.	Check Mean ± S.E.	
		3,963	4,141			
Prespray	*A. fumiferanae*			3.75 ± 0.45	2.91 ± 0.41	NS
	G. fumiferanae			3.14 ± .43	7.25 ± .55	S*
	P. hariolus			.04 ± .04	.04 ± .04	NS
	Tachinids			0	0	NS
	Others			.06 ± .42	.06 ± .06	NS
	Total			7.01 ± .65	10.25 ± .67	S*
		4,282	5,255			
Postspray 7-day	*A. fumiferanae*			7.13 ± .60	4.41 ± .44	S*
	G. fumiferanae			6.81 ± .74	13.29 ± .67	S*
	P. hariolus			0	.18 ± .09	S*
	Tachinids			.02 ± .02	.99 ± .21	S*
	Others			0	.25 ± .09	S*
	Total			13.99 ± 1.02	19.13 ± 1.00	S*
		3,257	5,643			
14-day	*A. fumiferanae*			8.53 ± .73	4.47 ± .52	S*
	G. fumiferanae			9.36 ± .86	17.14 ± .99	S*
	P. hariolus			0	.13 ± .05	S*
	Tachinids			.38 ± .12	2.57 ± .38	S*
	Others			.03 ± .03	.30 ± .12	S**
	Total			18.30 ± 1.11	24.63 ± 1.23	S*
		2,075	4,735			
21-day	*A. fumiferanae*			4.25 ± .74	1.63 ± .25	S*
	G. fumiferanae			11.71 ± 1.19	9.31 ± .67	S***
	P. hariolus			.03 ± .03	1.78 ± .36	S*
	Tachinids			.56 ± .22	2.29 ± .38	S*
	Others			.14 ± .10	.27 ± .13	NS
	Total	13,577	19,774	16.69 ± 1.31	15.33 ± 1.01	NS

*, **, *** Significant differences at .01, .05, and .10 respectively.

A. fumiferanae and *G. fumiferanae* to the fact that both parasites attack prewintering first instar budworm. Their development in the spring coincides with emergence of second instar budworm prior to treatment application. These parasitized larvae are photonegative and lack feeding stimuli characteristic of nonparasitized, photopositive larvae (Lewis 1960). Consequently, when pesticides are applied, parasitized larvae do not contact the pesticide as readily as nonparasitized larvae. The majority of other parasites attack late instar budworm which have ample opportunity to contact the pesticide. Hamel concluded that the significant difference is not attributed to effects of Dipel on parasites, but rather is a function of host response prior to and after spraying.

Spray deposit analysis.--Results of regression analysis show that the relation between log mg/m^2 and survival ratio was only significant in the Smith Creek spray block (Figure 5). When deposit (log $drops/cm^2$) was used in the regression as the dependent variable, the relation was nearly the same (Figure 6). Other spray blocks showed no significant relation between deposit and mortality. Perhaps if natural mortality had not masked the effect of deposit, a better relation could have been demonstrated. Table 6 shows deposit and population reduction by spray block.

Foliage protection was low in blocks sprayed with Dipel. Approximately 1.5 percent of the foliage was saved in Lime Creek block, 0.3 percent in Smith block, and 25 percent in Doe Creek block. Overall average of foliage saved was 5.6 percent.

Table 6.--Mean deposit and population reduction in *B.t.* sprayed blocks, Gallatin Ranger District, Gallatin National Forest, 1975.

Spray block	V.m.d.	Mg/m^2 a/	$Drops/cm^2$ a/	Percent population reduction
Lime	334.3	657.7	17.9	72.3
Doe	306.2	1,397.8	30.4	55.5
Smith	316.4	1,135.4	19.1	74.2

a/ Means were obtained by taking the highest value for one of four cards placed around each sample tree.

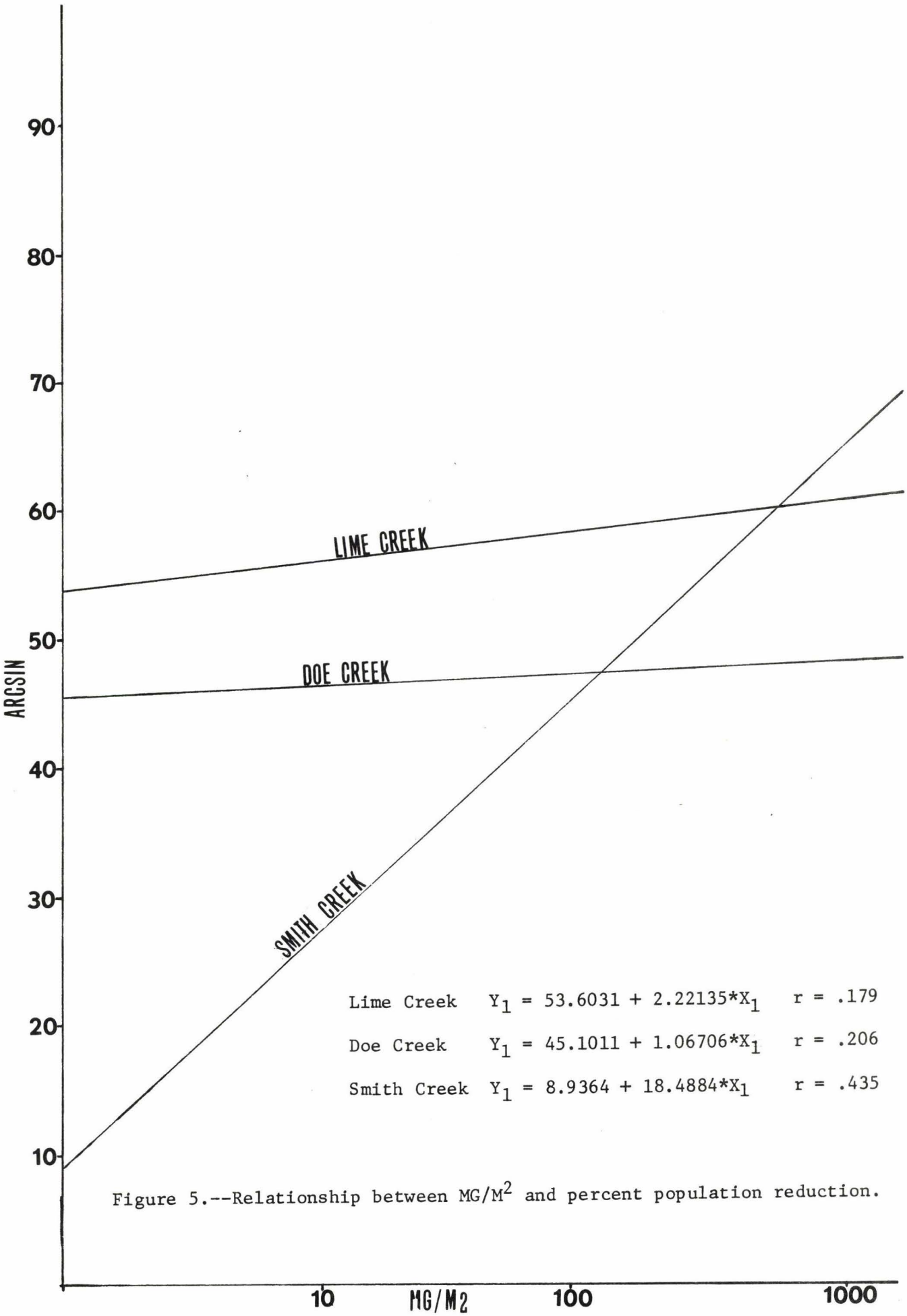


Figure 5.--Relationship between MG/M^2 and percent population reduction.

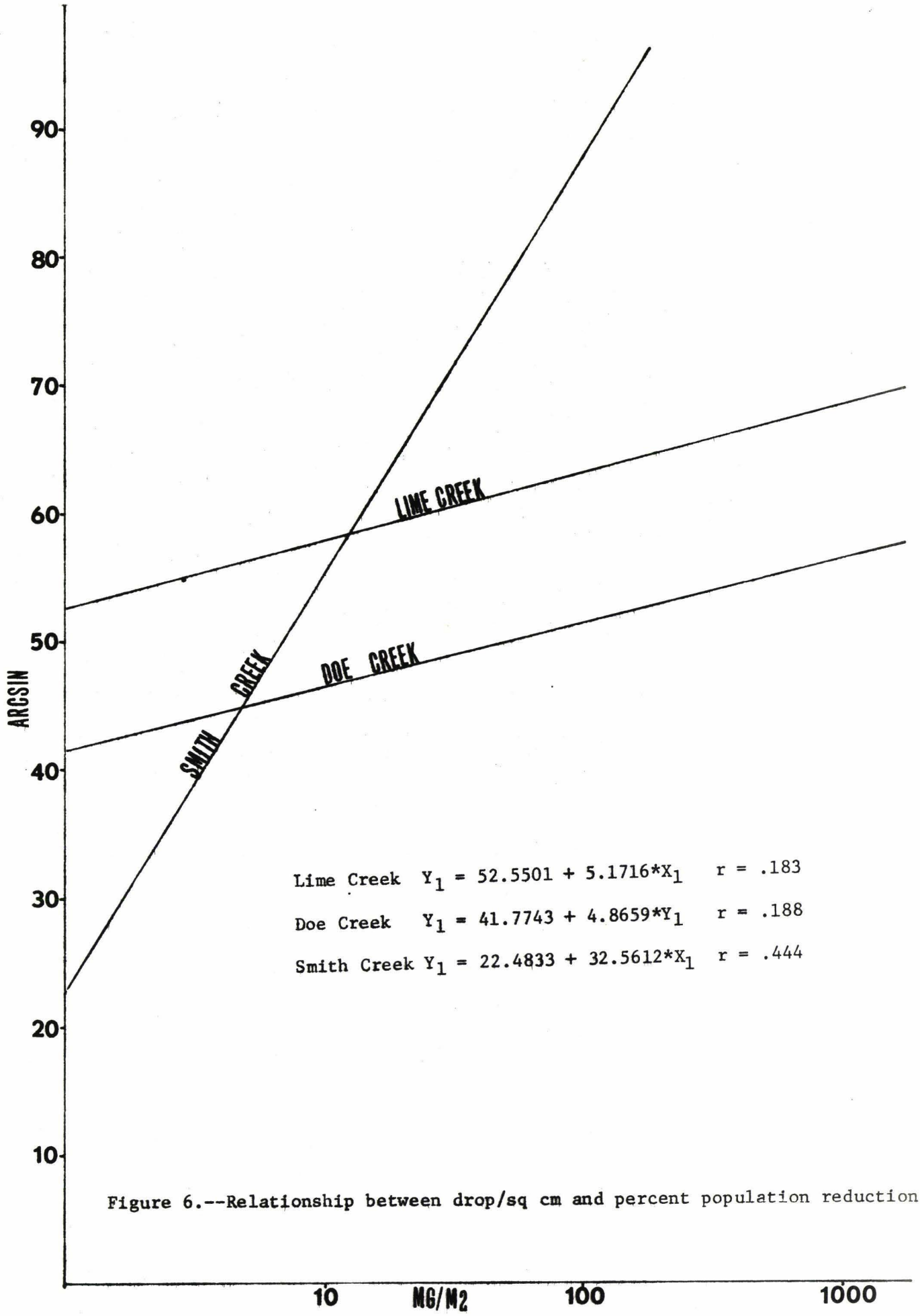


Figure 6.--Relationship between drop/sq cm and percent population reduction.

CONCLUSIONS

Dipel applied at 1 lb. in 2 gallons of water per acre provided up to 58 percent control against third and fourth instar populations of western spruce budworm. Foliage protection was 25 percent in one block and considered unsatisfactory. Significant parasite population disruptions occurred following spray application. Parasitism by *A. fumiferanae* and *G. fumiferanae* increased significantly from prespray to postspray. Parasitism by *P. hariolus*, *C. auricaudata* and *M. saundersii* decreased significantly from prespray to postspray. Correlation of spray deposit with larval mortality was statistically significant in only the Smith Creek block.

Because of the small size of spray blocks, inflight from adjacent nonsprayed stands, and small amount of foliage saved, we do not recommend resampling of the Gallatin spray and respective check blocks to determine larval populations or amount of defoliation in 1976. The manpower, money, and time required to resample these blocks would not be worth the effort. Reinfestation from adjacent infested stands will probably mask effects of 1975 treatments.

Bacillus thuringiensis is a unique pesticide in terms of its specificity, safety, and mode of action. Because of these highly desirable characteristics, it deserves increased attention from forest land managers.

On the basis of our results in attaining up to 74 percent population reduction, and reducing the population to 4.4 larvae/100 buds, we recommend that further testing be conducted so that *B.t.* can be considered as a viable alternative for reducing budworm populations and protecting foliage in high use sensitive areas in the future.

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